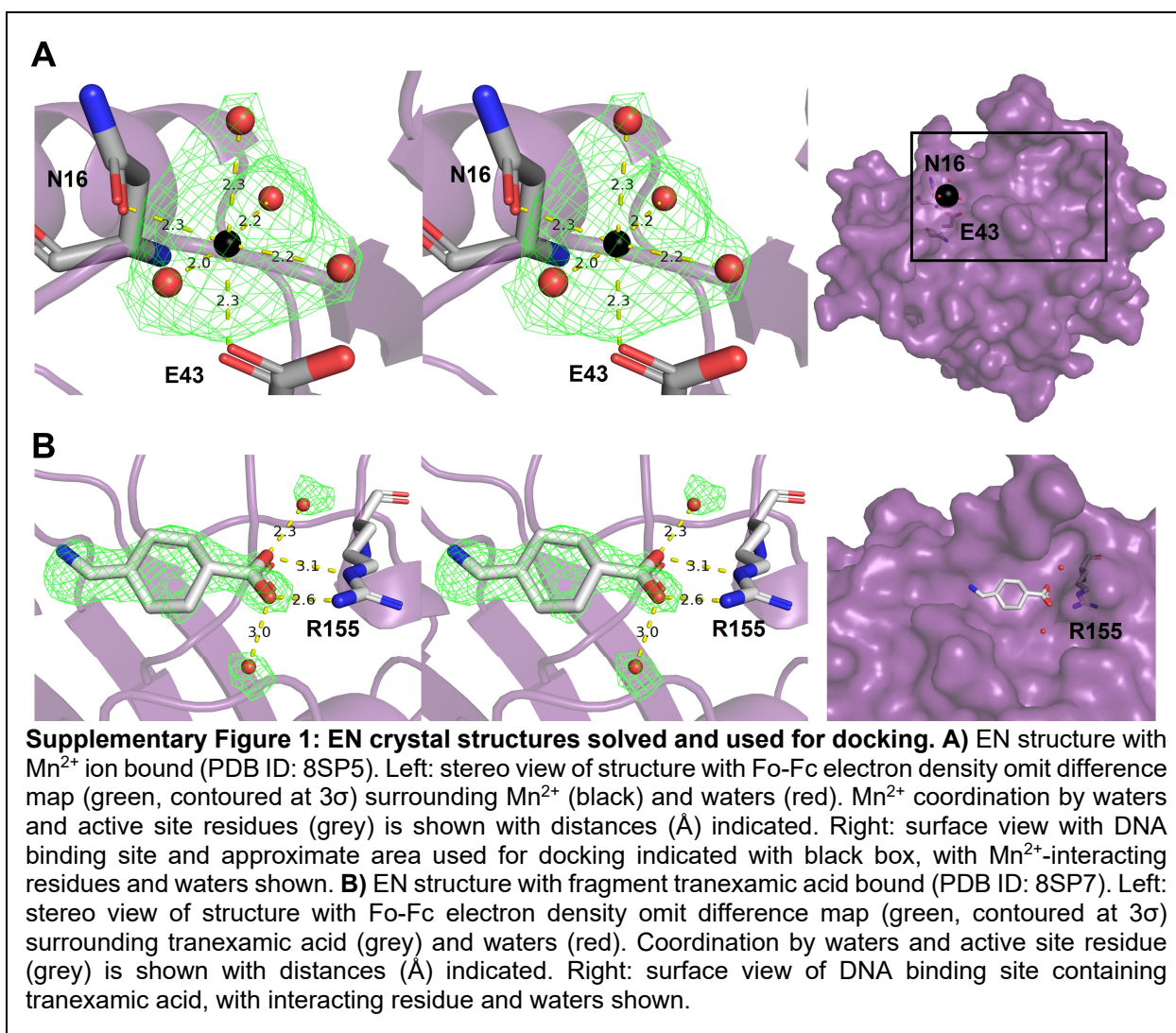
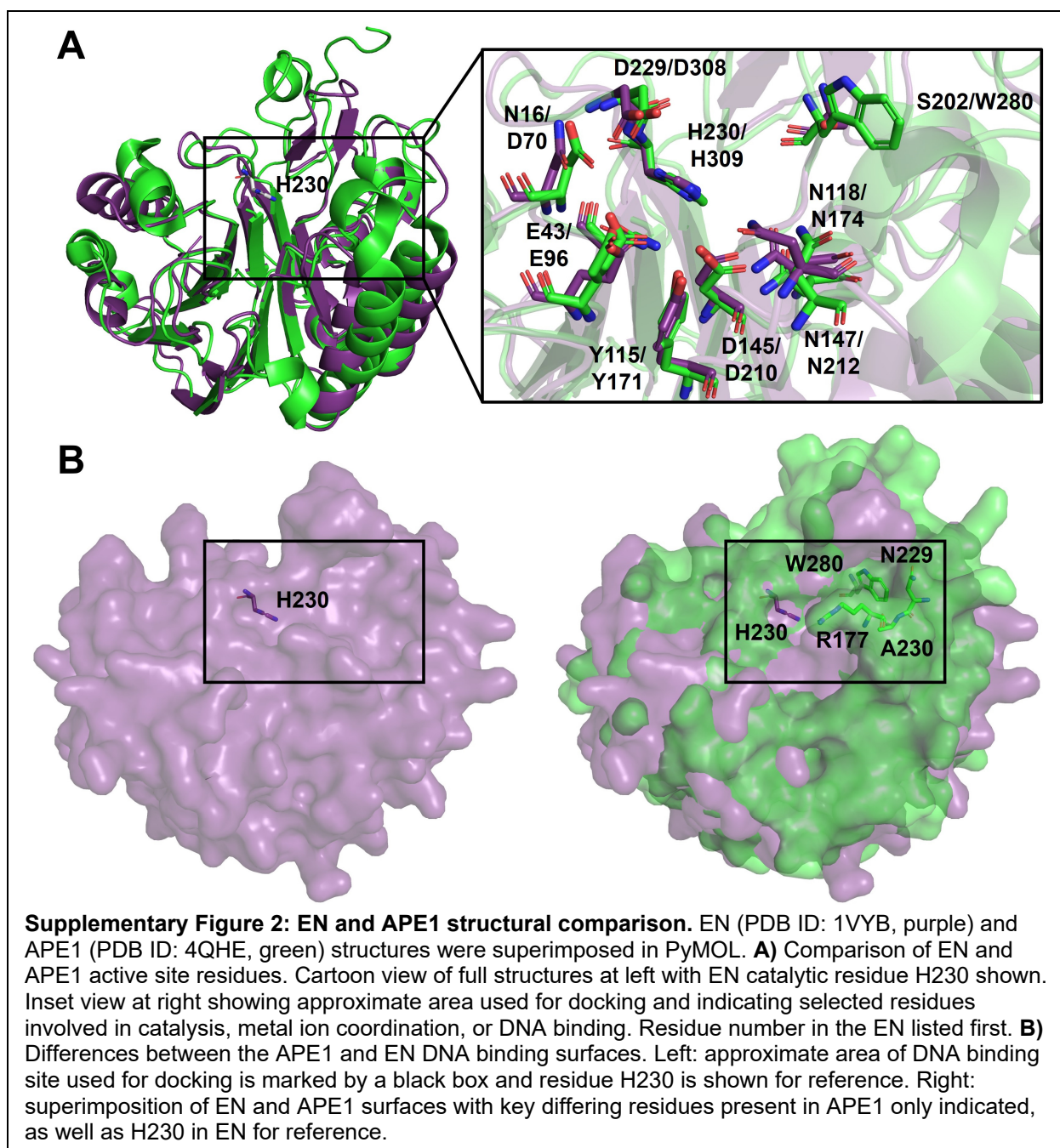


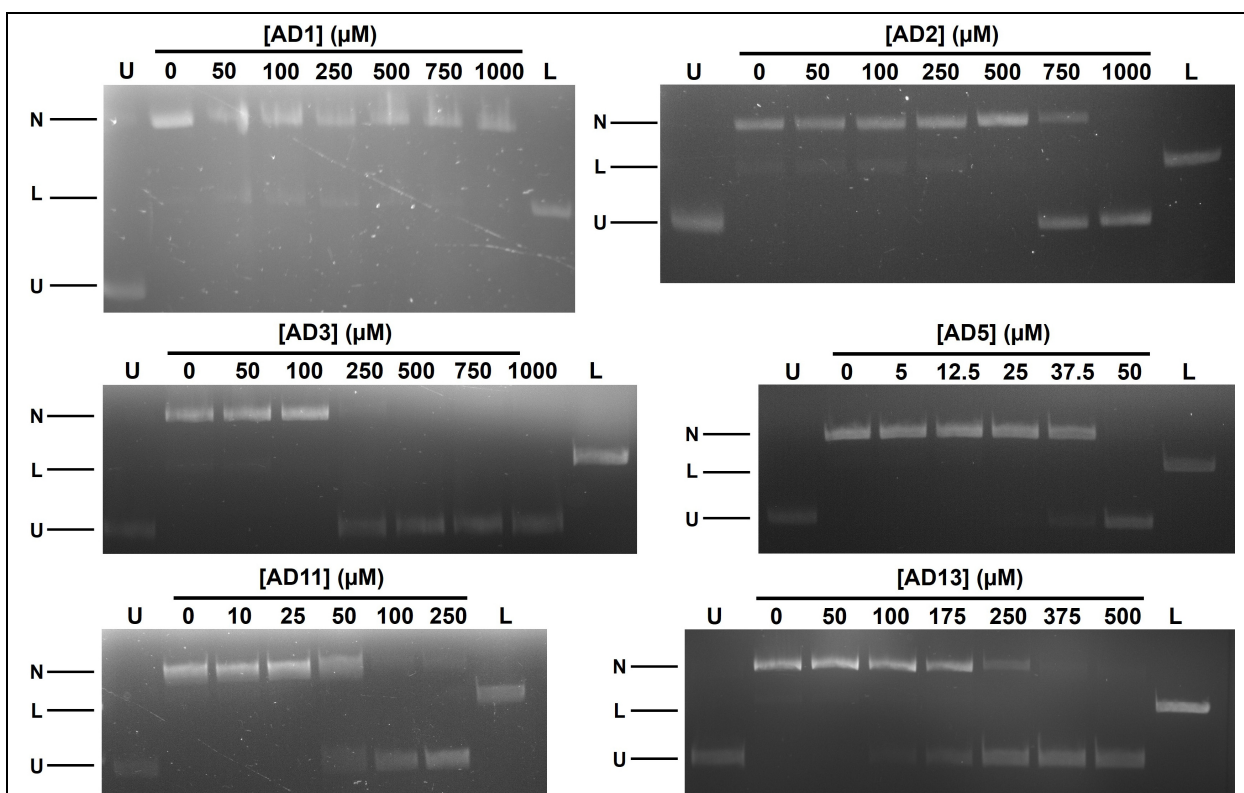
**Identification and characterization of small molecule inhibitors of the LINE-1
retrotransposon endonuclease**

Alexandra M. D'Ordine, Gerwald Jogl, John M. Sedivy

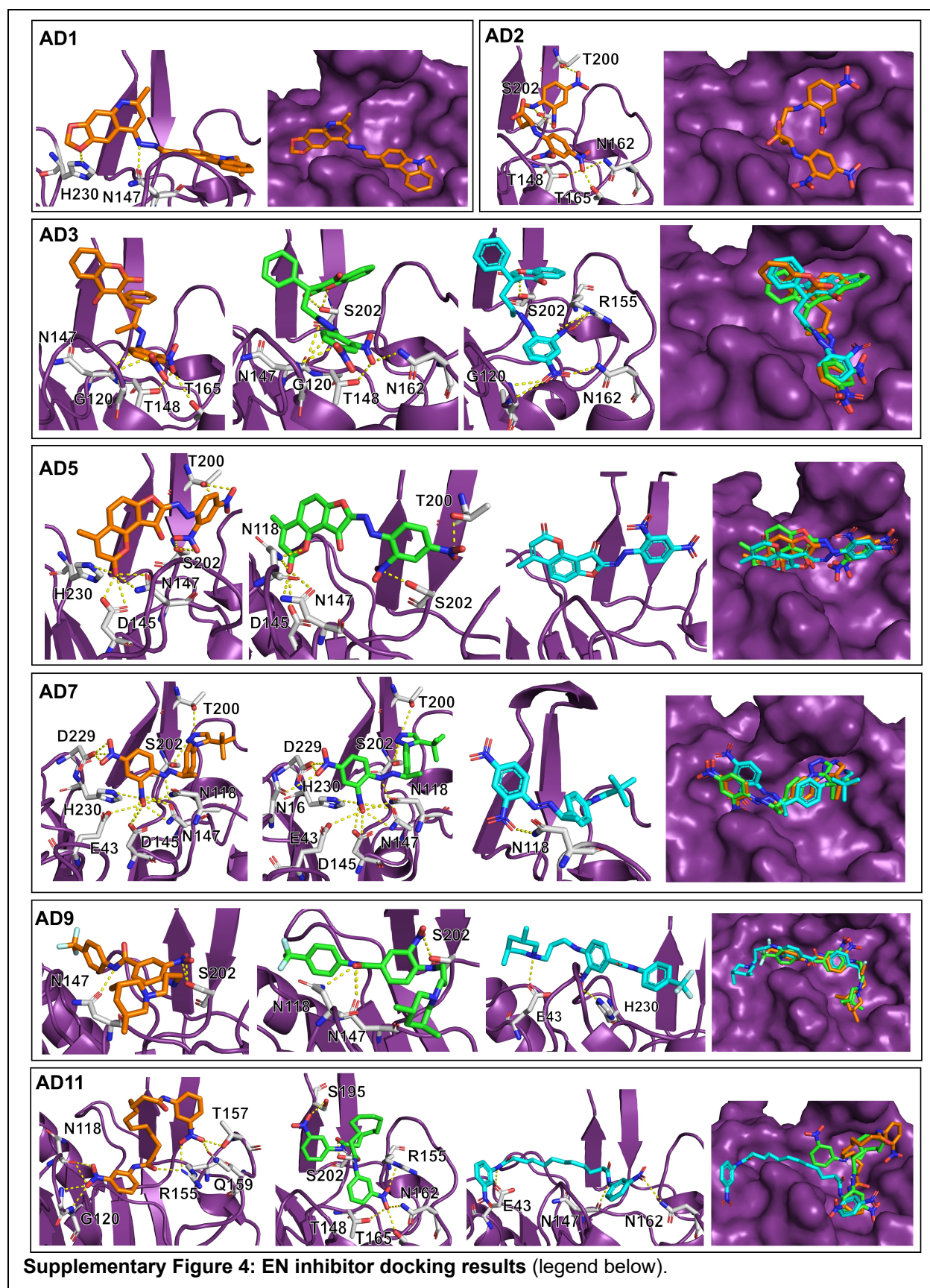
Supplementary Information

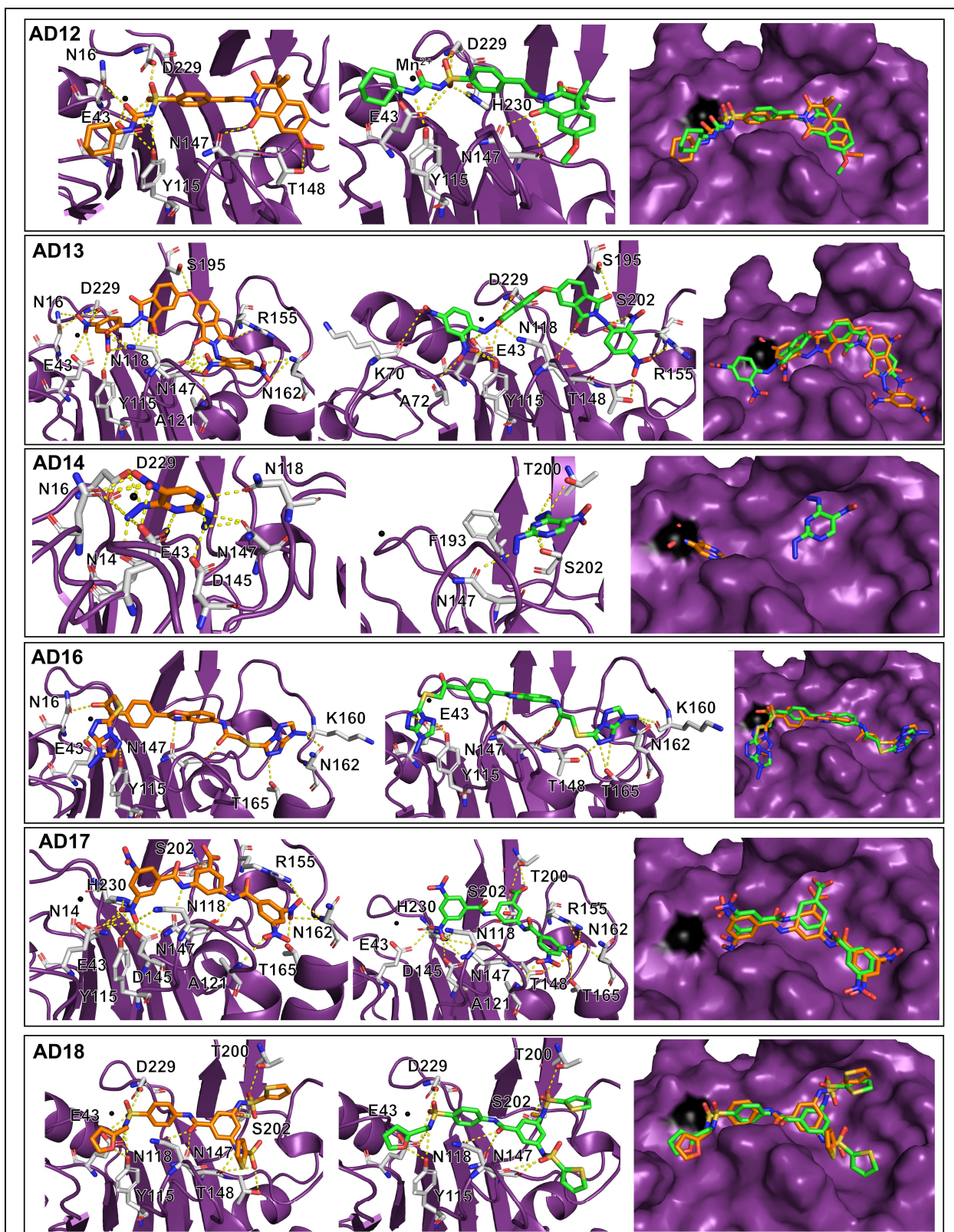




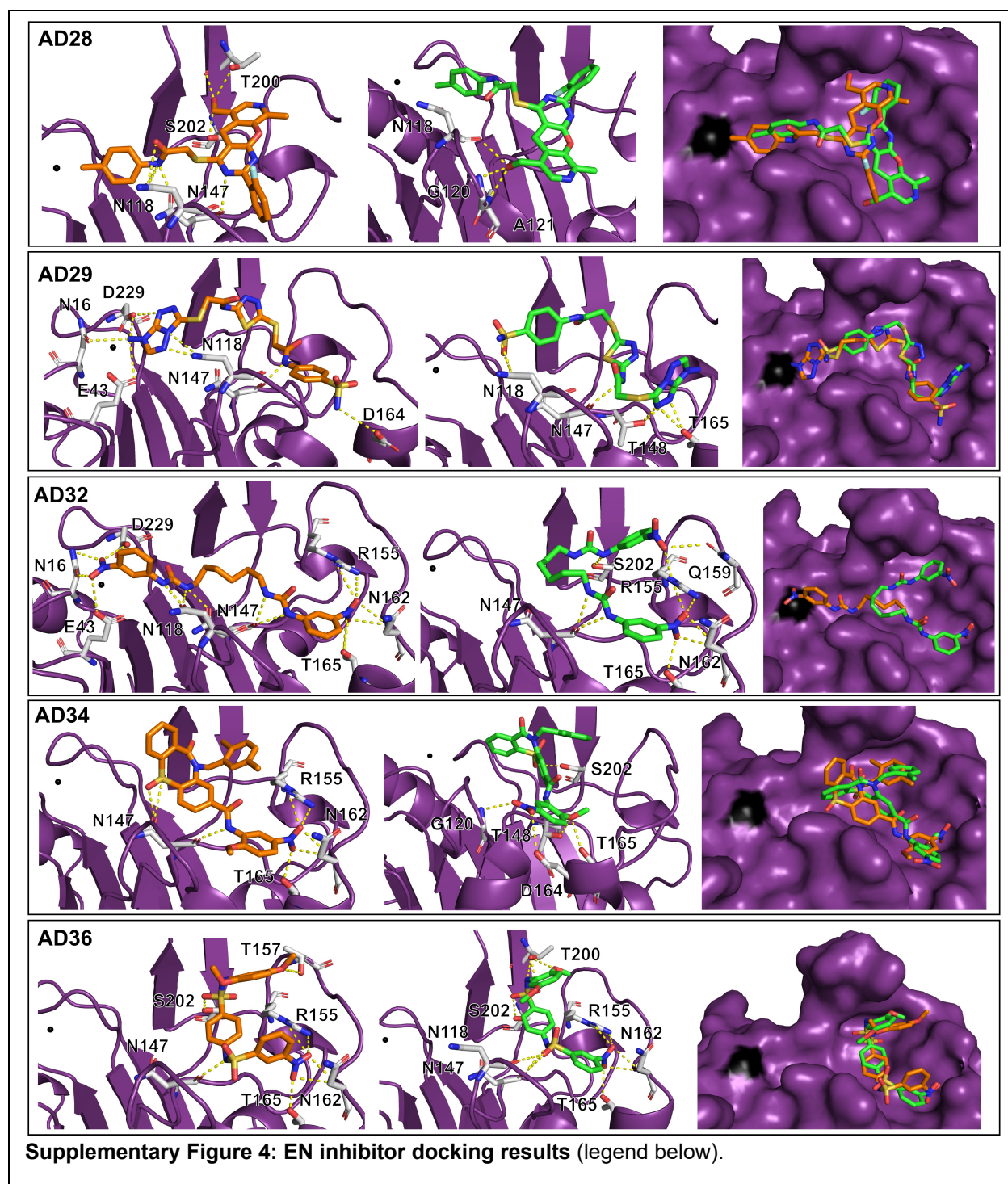


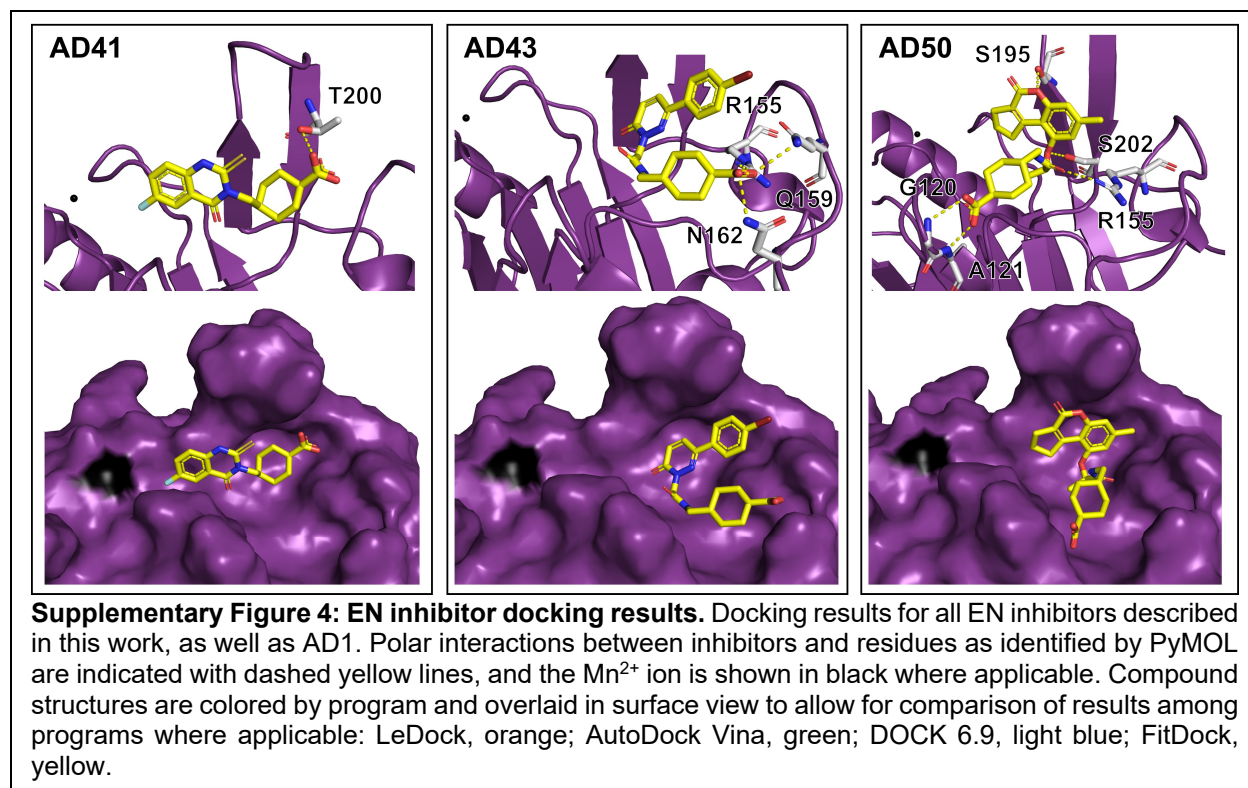
Supplementary Figure 3: Plasmid nicking assay results for selected compounds. Initial candidate compounds were screened using this previously developed EN activity assay. Activity is indicated by slower migration of plasmid on the agarose gel due to nicking and subsequent loss of supercoiling. U, uncut, supercoiled plasmid; L, linearized plasmid; N, nicked plasmid. Similar images were obtained from at least 2 independent experiments. Uncropped gel images are provided as a Source Data file.

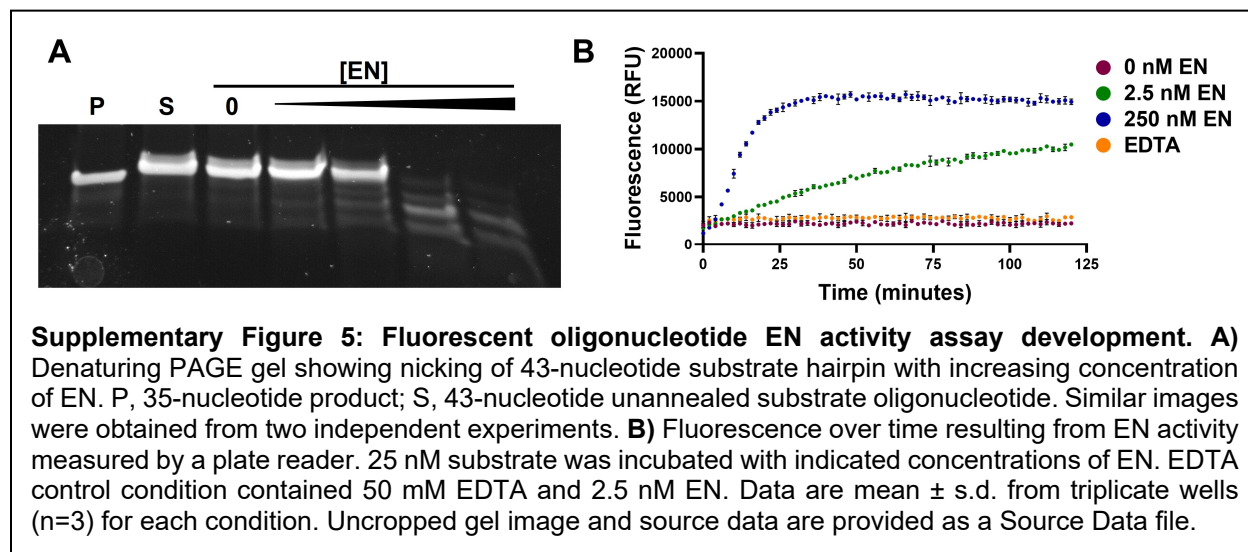




Supplementary Figure 4: EN inhibitor docking results (legend below).

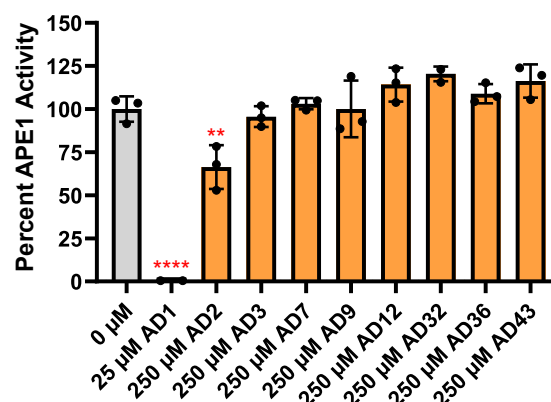




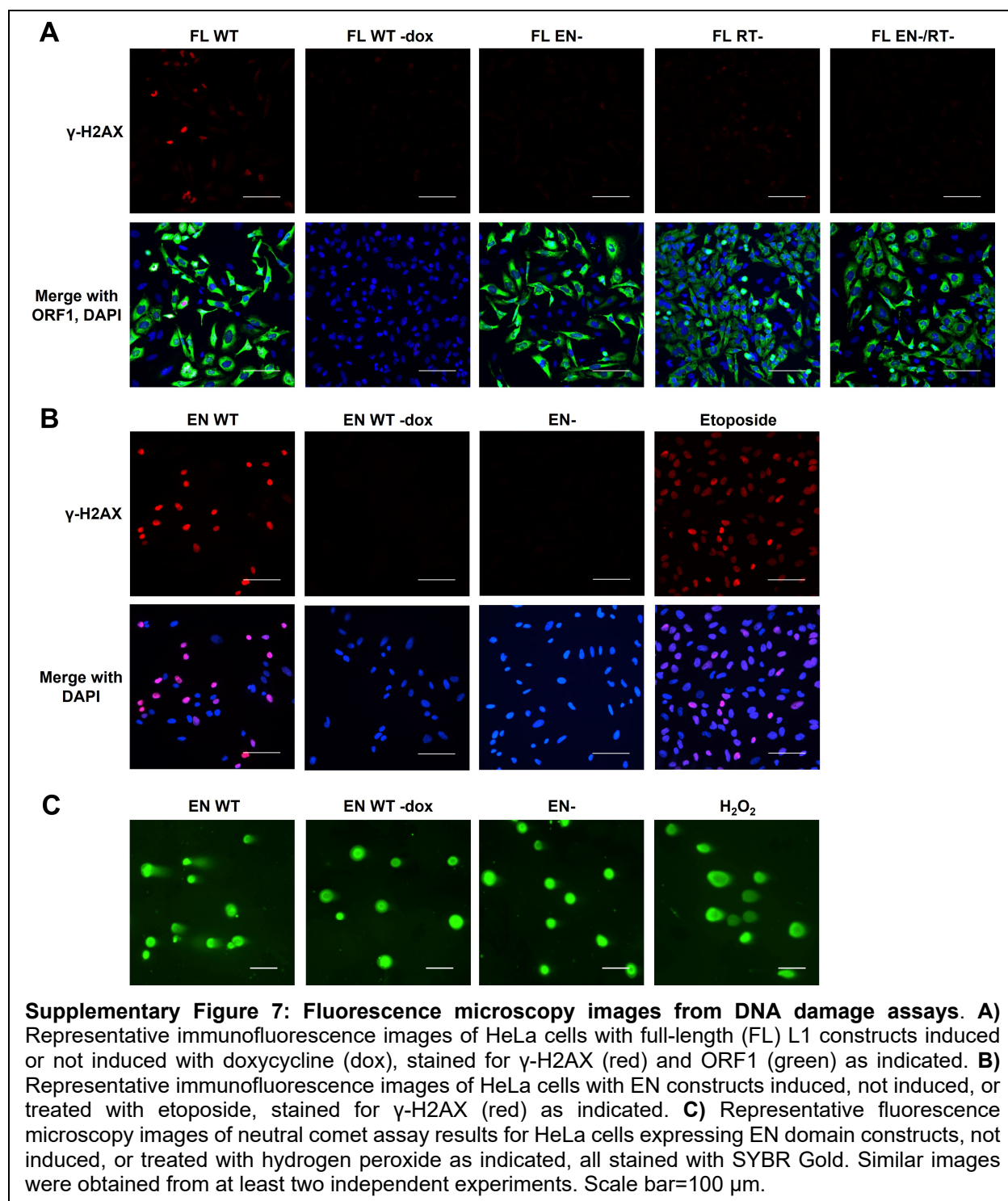


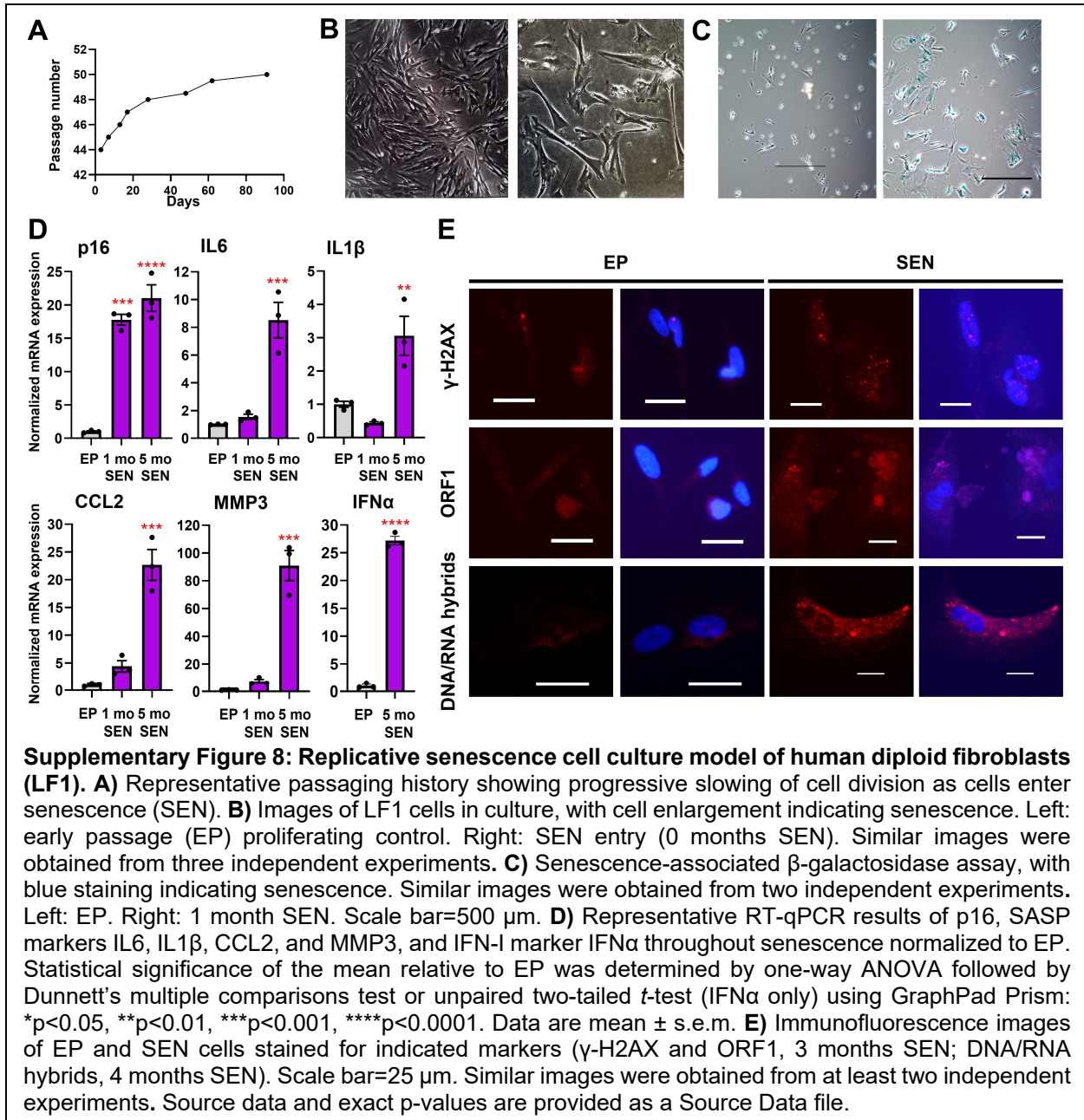
A

Name	APE1 activity (%)	Name	APE1 activity (%)	Name	APE1 activity (%)
AD1	0.5 ± 0.3	AD12	109.1 ± 5.2	AD29	81.2 ± 2.4
AD2	96.2 ± 3.4	AD13	84.4 ± 15.2	AD32	107.9 ± 2.8
AD3	103.5 ± 10.4	AD14	78.1 ± 6.6	AD34	113.8 ± 3.1
AD5	109.3 ± 1.9	AD16	95.5 ± 4.2	AD36	118.2 ± 11.9
AD7	118.8 ± 4.7	AD17	51.1 ± 8.7	AD41	111.5 ± 7.9
AD9	115.9 ± 3.1	AD18	103.1 ± 9.2	AD43	115.9 ± 7.4
AD11	79.4 ± 8.9	AD28	118.3 ± 1.2	AD50	115.7 ± 2.8

B

Supplementary Figure 6: APE1 assay testing EN inhibitors. A) Table of APE1 percent activities relative to 0 μM inhibitor control for inhibitors at 25 μM. Percent activity values were calculated from 3 independent experiments with 3 replicates each and are mean ± s.d. **B)** Percent activity of APE1 incubated with indicated inhibitors. Statistical significance of the mean relative to 0 μM inhibitor was determined by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are mean ± s.d (n=3 samples). Source data and exact p-values are provided as a Source Data file.





Supplementary Table 1: Data collection and refinement statistics for EN crystal structures^a

	EN complex with Mn ²⁺	EN complex with tranexamic acid
Data collection		
Space group	P 2 ₁ 2 ₁ 2	C 2 2 2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	93.10, 122.38, 43.41	54.22, 64.67, 132.36
α , β , γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	46.55-2.23 (2.30-2.23)	29.05-1.83 (1.87-1.83)
<i>R</i> _{merge}	0.195 (1.251)	0.088 (1.138)
CC _{1/2}	0.996 (0.853)	0.998 (0.897)
<i>I</i> / σ <i>I</i>	9.2 (2.2)	13.9 (2.1)
Completeness (%)	100 (100)	100 (100)
Multiplicity	13.5 (12.4)	13.6 (14.2)
Refinement		
Resolution (Å)	2.23	1.83
No. reflections	25007	20883
<i>R</i> _{work} / <i>R</i> _{free}	0.203/0.250	0.220/0.256
No. atoms		
Protein	3758	1819
Ligand/ion	27	30
Water	236	61
<i>B</i> -factors		
Protein	37.37	50.65
Ligand/ion	43.55	60.08
Water	38.09	51.24
R.m.s. deviations		
Bond lengths (Å)	0.007	0.006
Bond angles (°)	0.89	0.87
Ramachandran favored (%)	98.28	96.12
Ramachandran allowed (%)	1.72	3.45
Ramachandran outliers (%)	0.00	0.43
Rotamer outliers (%)	1.64	0.51

^a One crystal was used for each structure.

Supplementary Table 2: EN inhibitor and oligonucleotide binding by spectral shift. EN inhibitors were incubated with the substrate oligonucleotide used in the EN activity assay and labeled with Cy5. The change in spectral shift ratio between the oligonucleotide with and without each inhibitor (response amplitude), as well as the signal to noise ratio of this response, was measured and used by the MO.Control software (NanoTemper) to determine the presence or absence of binding. Inhibitors were tested at the highest soluble concentration in spectral shift assay buffer.

Compound	Response Amplitude	Signal to Noise Ratio	Binding Detected? (Y/N)
SYBR Gold	0.101	79.39	Y
AD2	0.003	1.811	N
AD3	0.004	0.373	N
AD5	0.009	3.487	N
AD7	0.009	2.553	N
AD9	0.003	0.398	N
AD11	0.012	1.936	N
AD12	0.014	3.485	N
AD13	0.005	1.436	N
AD14	0.012	1.282	N
AD16	0.019	8.275	Y
AD17	0.004	2.000	N
AD18	0	0.170	N
AD28	0.004	2.501	N
AD29	0.006	2.466	N
AD32	0.001	0.839	N
AD34	0.001	0.667	N
AD36	0.014	4.209	N
AD41	0.001	0.865	N
AD43	0.017	2.642	N
AD50	0.003	2.854	N

Supplementary Table 3: Summary of DNA damage assay replicates. Summary of independent experiments of γ -H2AX and comet assays (Fig. 5) reporting means of γ -H2AX intensities or comet tail lengths of individual nuclei (n) normalized to WT. Average intensity or tail length and associated standard deviation across replicates is also shown. Statistical significance of the mean relative to WT was calculated by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism. Source data are provided as a Source Data file.

FL γ-H2AX	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Summary	
Treatment	mean	p-value	n	mean	p-value	n	mean	p-value	n	mean	p-value	n	average	s.d.
WT	1		117	1		83	1		143	1		120	1	
-dox	0.421	<0.0001	15	0.595	<0.0001	52	0.613	<0.0001	130	0.543	<0.0001	16	0.543	0.087
EN-	0.395	<0.0001	21	0.703	<0.0001	46	0.644	<0.0001	197	0.743	0.0302	15	0.621	0.156
RT-	0.453	<0.0001	80	0.634	<0.0001	280	0.598	<0.0001	325	0.657	<0.0001	67	0.586	0.091
EN-/RT-	0.361	<0.0001	41	0.588	<0.0001	113	0.567	<0.0001	413	0.602	<0.0001	26	0.529	0.113
10 μ M 3TC	0.742	<0.0001	168	0.671	<0.0001	71	0.740	<0.0001	173				0.718	0.040
20 μ M AD3	0.752	<0.0001	104	0.782	0.0006	38	0.727	<0.0001	336				0.754	0.028
20 μ M AD7	0.673	<0.0001	107	0.982	>0.9999	107	0.987	>0.9999	197				0.881	0.180
50 μ M AD11				0.738	<0.0001	62	0.875	0.0009	151	0.790	0.0006	56	0.801	0.069
50 μ M AD12	0.742	<0.0001	120	0.704	<0.0001	25	0.777	<0.0001	155				0.741	0.037
50 μ M AD14	0.740	0.0005	62	0.670	<0.0001	85	0.909	0.0181	215				0.773	0.123
50 μ M AD16	0.788	0.0349	41				0.872	0.0002	220	0.967	0.9719	169	0.876	0.090
50 μ M AD29				0.655	<0.0001	64	0.742	<0.0001	199	0.722	<0.0001	116	0.707	0.046
5 μ M AD32				0.822	0.0008	68	0.788	<0.0001	195	0.922	0.6214	58	0.844	0.070

EN γ-H2AX	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Replicate 5			Summary	
Treatment	mean	p-value	n	mean	p-value	n	mean	p-value	n	mean	p-value	n	mean	p-value	n	average	s.d.
WT	1	N/A	257	1	N/A	301	1	N/A	257	1	N/A	214	1	N/A	544	1	
no dox				0.337	<0.0001	200	0.461	<0.0001	619	0.365	<0.0001	346	0.207	<0.0001	804	0.342	0.105
EN-	0.292	0.0096	42	0.350	<0.0001	363	0.083	<0.0001	323	0.345	<0.0001	442	0.193	<0.0001	668	0.253	0.114
20 μ M AD3	0.769	0.8621	51	0.730	<0.0001	135	0.624	<0.0001	174							0.708	0.075
20 μ M AD7	0.630	0.2813	67	0.830	0.0116	117							0.852	0.0063	322	0.771	0.122
50 μ M AD11	0.637	0.359	57	0.615	<0.0001	109	0.633	<0.0001	106							0.628	0.011
100 μ M AD12	0.522	0.1429	48	0.883	0.1349	142	0.327	<0.0001	136							0.577	0.282
50 μ M AD14	0.779	0.7691	88	0.662	<0.0001	116	0.414	<0.0001	133							0.618	0.187
25 μ M AD16	0.656	0.3427	71	0.791	<0.0001	204	0.325	<0.0001	60							0.591	0.240
100 μ M AD29	0.409	0.0308	50	0.534	<0.0001	75	0.903	0.7755	126				0.691	<0.0001	242	0.634	0.213
5 μ M AD32				0.516	<0.0001	111	0.604	<0.0001	160	0.604	<0.0001	159				0.574	0.051

EN comet	Replicate 1			Replicate 2			Replicate 3			Replicate 4			Summary	
Treatment	mean	p-value	n	mean	p-value	n	mean	p-value	n	mean	p-value	n	average	s.d.
WT	1		54	1		117	1		62	1		46	1	
no dox	0.661	0.0484	56	0.612	0.0019	127	0.39	<0.0001	90	0.326	<0.0001	83	0.498	0.163
EN-	0.643	0.0238	67	0.532	<0.0001	166	0.36	<0.0001	82	0.559	0.0003	76	0.524	0.117
20 μ M AD3				0.679	0.0328	98	0.57	0.0062	59	0.482	0.0005	34	0.576	0.099
20 μ M AD7				0.573	0.0009	107	0.81	0.6224	59	0.310	<0.0001	48	0.566	0.252
50 μ M AD11	0.605	0.0364	37	0.832	0.6311	90	0.81	0.6442	52	0.384	<0.0001	40	0.658	0.209
50 μ M AD12				0.976	>0.9999	103	0.85	0.8869	47	0.628	0.023	37	0.819	0.176
50 μ M AD14	0.589	0.0237	39	0.936	0.9985	106	0.58	0.006	68	0.644	0.0264	41	0.688	0.168
25 μ M AD16	0.876	0.899	32	0.679	0.0261	108	0.59	0.0076	67	0.505	0.0001	54	0.662	0.159
50 μ M AD29				0.646	0.0133	98	0.48	0.0002	68	0.544	0.0005	56	0.556	0.085
25 μ M AD32				1.003	>0.9999	83	0.61	0.014	66	0.564	0.0202	23	0.726	0.241